Environmental and Experimental Botany 155 (2018) 695–701

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Plant drought resistance is mediated by soil microbial community structure and soil-plant feedbacks in a savanna tree species

Nianxun Xi[a,b], Chengjin Chu[a,*], Juliette M.G. Bloor[c]

[a] Department of Ecology, State Key Laboratory of Biocontrol and School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China
[b] Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun 666303, Yunnan, China
[c] INRA, VetAgro-Sup, UREP (Grassland Ecosystem Research Unit), 5 Chemin de Beaulieu, F-63100 Clermont-Ferrand, France

ARTICLE INFO

Keywords:
Climate change
Phospholipid fatty acid analysis
Plant functional traits
Plant-soil (below-ground) interactions
Soil microbes
Savanna ecosystems

ABSTRACT

Soil microbial communities have the potential to modify plant performance and condition plant species responses to environmental change, but the role of soil microorganisms for plant drought responses remains unclear. We used a novel experimental approach to examine the interactive effects of drought and presence of soil microbes on biomass production and plant traits in a savanna tree species. Seedlings of *Bauhinia brachycarpa* were grown in sterilized or 'live' soil, with or without drought, during a 24-week greenhouse experiment. Soil microbial community structure was assessed with phospholipid fatty acid analysis and soil-plant feedback effects were measured. Both drought and the presence of soil microbes decreased plant growth and biomass produced per gram nitrogen (a proxy for N use efficiency) but increased biomass allocation to roots. However, the presence of soil microbes increased plant drought resistance, driven by weakened soil-plant feedbacks under dry conditions. Experimental drought was associated with an increase in the Gram positive: Gram negative bacteria ratio, but did not affect the fungi: bacteria ratio or total microbial biomass. Our results suggest that soil microbes mediate plant responses to drought via soil-plant feedbacks and drought-induced changes in microbial community structure. These findings highlight the importance of plant-soil interactions for improved mechanistic understanding of savanna function, and confirm that characteristics of the soil microbial community could have significant implications for ecosystem stability in a changing environment.

1. Introduction

Savannas are a widespread and diverse tree-grass ecosystem, providing important services such as carbon sequestration, livestock production and biodiversity (Scholes and Archer, 1997; Baudena et al., 2015). Within these systems, changes in tree cover can have significant consequences for ecosystem function and earth-atmosphere feedbacks (Druce et al., 2008; Baudena et al., 2015). Along with fires and land-use change, water availability is considered to be a key driver of tree abundance in savanna systems (Bond, 2008). As precipitation patterns are expected to shift in the future (IPCC, 2007), understanding the mechanisms of drought tolerance in savanna tree species is central to predicting future savanna ecosystem function and resilience to climate change (Craine et al., 2013).

Impacts of drought on plant physiology and growth are well documented, and numerous studies have examined the role of plant traits for plant drought tolerance (Chaves et al., 2003; van der Molen et al., 2011; Zwicke et al., 2015). However, growing evidence suggests that plant species responses to environmental change may also be mediated by soil microorganisms (Yang et al., 2009; Bloor and Bardgett, 2012; Fuchslueger et al., 2014; Kannenberg and Phillips, 2017; Fry et al., 2018). Soil microbial communities have the potential to influence plant growth and functional traits via changes in soil properties and the accumulation of soil pathogens or mutualists (Friesen et al., 2011; Smith-Ramesh and Reynolds, 2017). Although soil feedback effects are often negative, microbial effects on plants can also be neutral or positive depending on the different microbial groups involved (Kulmatiski et al., 2008; van der Putten et al., 2013). For example, mycorrhizal fungi may be of benefit to water-stressed plants by increasing access to soil water, improving plant hydraulics and gas exchange, whereas fungal pathogens may exacerbate plant vulnerability to drought (Aroca and Ruiz-Lozano, 2009; Kannenberg and Phillips, 2017). Disentangling the contribution of soil microorganisms for plant drought responses is complicated as it requires the use of sterile growing medium in experiments. To date, the majority of studies on plant responses to drought have been carried out in the presence of soil microbes (but see Kannenberg and

* Corresponding author.
E-mail address: chuchjin@mail.sysu.edu.cn (C. Chu).

https://doi.org/10.1016/j.envexpbot.2018.08.013
Received 21 May 2018; Received in revised form 25 July 2018; Accepted 12 August 2018
Available online 15 August 2018
0098-8472/ © 2018 Elsevier B.V. All rights reserved.

Table 1
Effects of water treatments, soil treatments and their interaction on plant biomass and functional traits.

		Water treatment		Soil treatment		Water × soil	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Total biomass	1, 20	169.7	< 0.001	315.2	< 0.001	49.79	< 0.001
Relative growth rate	1, 20	109.56	< 0.001	230.45	< 0.001	7.06	0.015
Shoot biomass	1, 20	67.02	< 0.001	29.28	< 0.001	12.69	0.002
Root: shoot ratio	1, 20	18.00	< 0.001	22.31	< 0.001	2.33	0.142
Specific leaf area	1, 20	0.66	0.426	0.75	0.396	2.10	0.163
Specific root length	1, 20	1.34	0.260	22.43	< 0.001	0.13	0.727
Plant N content	1, 20	82.36	< 0.001	50.90	< 0.001	0.86	0.366
Biomass: N ratio	1, 20	0.37	0.548	32.67	< 0.001	12.28	0.002

Phillips, 2017), and thus the importance of soil microbial communities for plant sensitivity to environmental fluctuations remains poorly understood.

Soil microbial effects on plants may be driven by microbial activity and microbial community structure, both of which are under the influence of abiotic factors (Lau and Lennon, 2011; van der Putten et al., 2016). In the case of drought, reduced soil water availability may directly depress soil microbial activity and biomass production (Schimel et al., 2007). Moreover, soil microbial communities may change under dry conditions due to the selection of drought-tolerant groups such as fungi (with lower nutrient requirements and a higher water acquisition capacity) or Gram + bacteria (with a thicker peptidoglycan cell wall layer) (Williams and Rice, 2007; Manzoni et al., 2012). In theory, drought-induced shifts in soil microbial activity and community structure should modify plant-soil interactions for nitrogen (N) (Bloor and Bardgett, 2012) and the relative abundance of pathogenic fungi (van der Putten et al., 2013), with cascading effects on soil-plant feedbacks. In practice, few studies have simultaneously investigated above- and belowground responses to water availability and the consequences of soil-plant interactions for plant resistance to drought (Karlowsky et al., 2017).

Here we examine the effects of reduced water availability on plant growth and functional traits in seedlings of a savanna tree species, *Bauhinia brachycarpa*, growing in sterilized or unsterilized soil medium. The main objective was to investigate the linkages between plant drought resistance, soil microbial community structure and soil-plant feedbacks. We focus on the seedling stage since this is a bottleneck for successful tree recruitment in natural communities, and woody seedlings are known to show high sensitivity to biotic and abiotic conditions (Bloor et al., 2009; Comita et al., 2014). We hypothesize that: 1) presence of soil microbes has negative effects on plant growth; 2) plant drought resistance is modified in the presence of soil microbes. We also assess whether drought alters soil feedback effects due to drought-induced changes in either microbial biomass or community structure.

2. Material and methods

2.1. Experimental design

The greenhouse experiment was performed at the Xishuangbanna tropical botanical garden, Chinese Academy of Sciences, China (21°41′N, 101°25′E, 570 a.s.l.). The experiment consisted of two treatments in a factorial design: soil treatment ('live', sterile) and water treatment (wet, dry). Each of the four treatment combinations was replicated six times.

In May 2016, we collected *Bauhinia brachycarpa* seeds in a nearby savanna ecosystem (23°28′N, 102°10′E, 480 m a.s.l.); *Bauhinia brachycarpa* is a drought-tolerant species with small leaves and deep roots that dominates in the dry savanna ecosystem of the Yuanjiang valley, Yunnan, China. Seeds were surface-sterilized (1 min 75% ethanol, 3 min 2% NaOCl, 1 min 75% ethanol, 3 min distilled water), germinated in plug trays filled with sterilized sands, and left to grow for two weeks. One week prior to seedling transplanting, field soil in the 0–15 cm soil layer was collected beneath mature *B. brachycarpa* trees in the Yuanjiang savanna ecosystem. Field soil was pooled and homogenized to avoid effects of soil heterogeneity. Half of the soil was kept at 5 °C whereas the remainder was steam-sterilized for 3 h at 121 °C and then kept at 5 °C prior to the start of the experiment.

Pots (14 cm diameter × 16 cm height) were filled with a sand, peat and soil mix (volume ratio of 6: 3: 1). All sand and peat used in the pots was steam-sterilized and soil used was either sterilized field soil (sterile soil treatment) or live field soil (live soil treatment). On 27 August 2016, one tree seedling was transplanted into each pot, and all seedlings were left to grow with regular watering in the greenhouse conditions for nine weeks. Pots were randomly located in the greenhouse and re-arranged weekly to avoid possible positioning effects. From 29 October 2016 onwards, seedlings allocated to the 'wet' treatment received water addition based on the precipitation regime in the Yuanjiang savanna ecosystem during the wet season, whereas seedlings in the 'dry' treatment had an 85% reduction in watering regime, equivalent to the average precipitation regime during the dry season. In the 'wet' treatment, pots received two or three watering events per week, resulting in a total water addition of 348 ml (equivalent to the average rainfall of 589.9 mm in the wet season i.e. May–October, based on long-term weather records). 'Dry' treatments received 61 ml of water weekly (equivalent to the average rainfall of 104.1 mm in the dry season i.e. November–April). Plants were left to grow for a further 15 weeks under these watering regimes. Over the total experimental period, the average air temperature was 24 °C, and the average air humidity was 67% (consistent with long-term weather records for the Yuanjiang valley).

2.2. Plant sampling and calculations

Initial seedling dry mass was determined at the date of seedling transplantation for 13 individual seedlings by harvesting, oven-drying (70 °C for 72 h) and then weighing. All remaining experimental plants were harvested in mid-February 2017 and separated into shoots and roots. Roots were washed to remove soil and organic debris. Specific leaf area (SLA, the one-sided area of a fresh leaf divided by its oven-dry mass, $m^2 g^{-1}$) and specific root length (SRL, the ratio of root length to dry mass of fine roots, $m\ g^{-1}$) were determined following standard protocols (Pérez-Harguindeguy et al., 2013). We chose specific leaf area and specific root length, as they are key plant traits in predicting plant-soil feedbacks (Baxendale et al., 2014; Cortois et al., 2016). SLA was measured on 2–4 fully expanded fresh leaves per individual. Leaf area was determined using ImageJ software after scanning with a flatbed scanner (Canon Lide 120, Canon, Japan). SRL was measured on a subsample of fine fresh roots per individual. Root length was measured using SmartRoot software (Lobet et al., 2011) and a flatbed scanner (Canon Lide 120, Canon, Japan). All shoots and roots were then oven-dried at 70 °C for 72 h to determine dry mass. Shoot biomass, root biomass and total biomass were used as measures of plant growth. Dried shoot and root material were ground and analysed for whole-

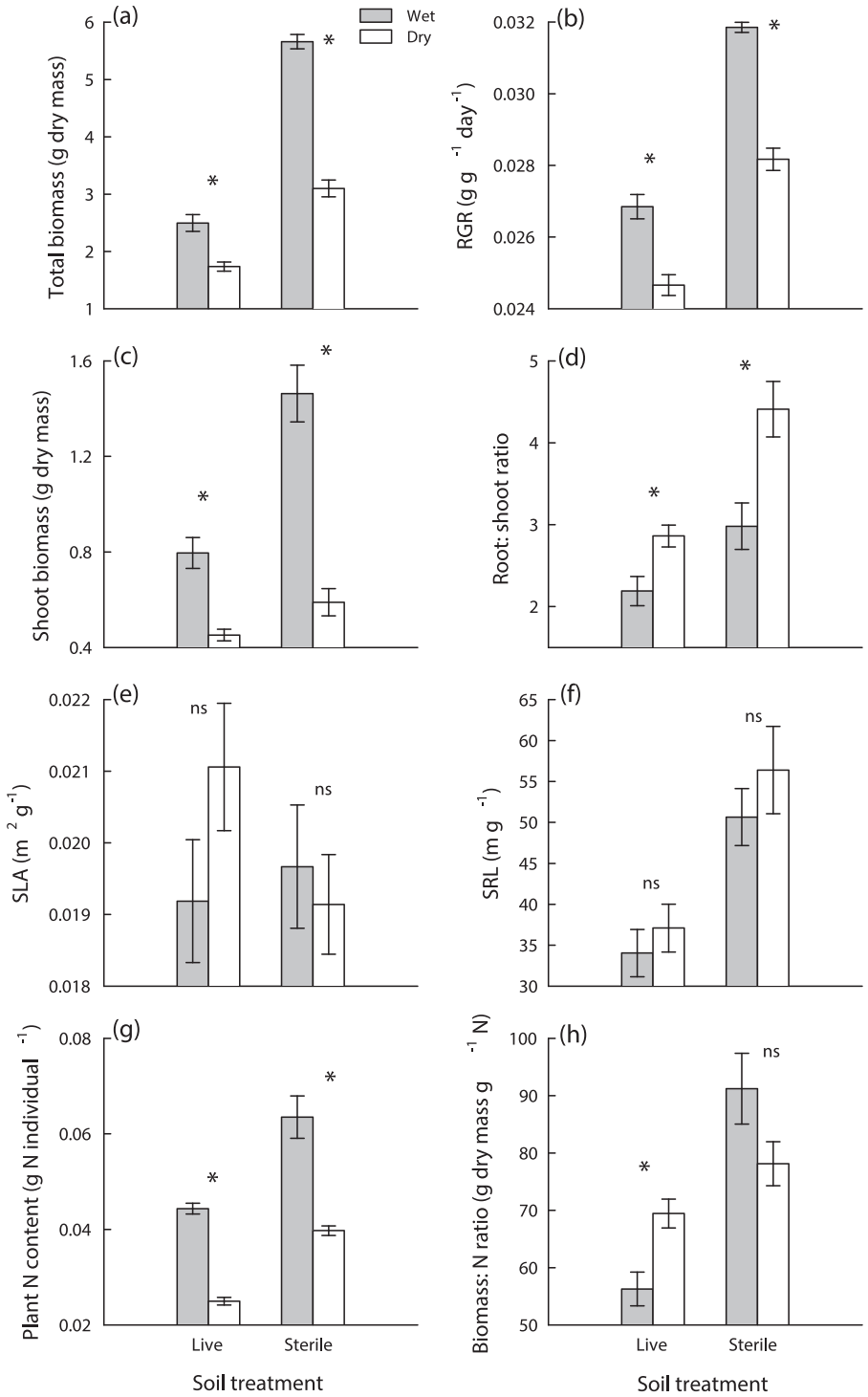

Fig. 1. Effects of water and soil treatments on (a) total biomass, (b) relative growth rate, (c) shoot biomass, (d) root: shoot ratio, (e) specific leaf area, (f) specific root length, (g) plant N content and (h) biomass: N ratio. Mean ± SE are shown (n = 6). Differences between wet and dry treatments are indicated (ns, $p > 0.05$; *, $p < 0.05$).

plant level nitrogen content using an elemental analyser (Vario isotope cube, Elementar Analysensysteme GmbH, Germany). The ratio of whole-plant biomass to N content (g dry mass g^{-1} N) was assessed as proxy of N use efficiency (Fargione and Tilman, 2006; Xi et al., 2015).

Root: shoot ratio was calculated by dividing the root biomass by the shoot biomass. Relative growth rate (RGR, g g^{-1} day^{-1}) was assessed over the total experimental period for each individual plant i.e. from seedling transplantation to final harvest. RGR of 24-week-old plants was calculated as the difference between their natural-logged mass (lnM_{t1}) and the average value of natural-logged initial masses of individual seedlings ($\overline{lnM_{t0}}$), all divided by the interval of growth (t_1-t_0, days) (Hoffmann and Poorter, 2002):

$$RGR = \frac{lnM_{t1} - \overline{lnM_{t0}}}{t_1 - t_0}$$

Effects of water availability on plant biomass were assessed for live or sterile soil as:

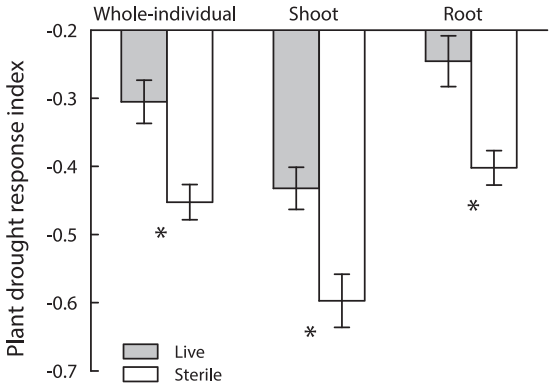

Fig. 2. Plant drought response index of total, shoot and root biomass in live or sterile soil treatments. Mean ± SE are shown (n = 6). Asterisks indicate significant differences between live and sterile treatments ($p < 0.05$).

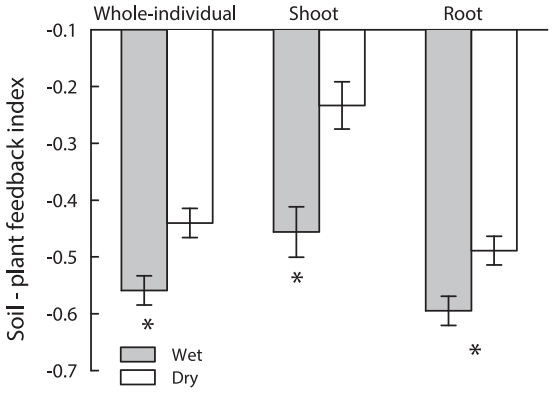

Fig. 3. Soil – plant feedback index of total, shoot and root biomass in dry or wet treatments. Mean ± SE are shown (n = 6). Asterisks indicate significant differences between wet and dry treatments ($p < 0.05$).

$$Plant\ drought\ response\ index = \frac{M_{dry} - \overline{M_{wet}}}{\overline{M_{wet}}}$$

where M_{dry} is the mass of individuals in dry treatments, and $\overline{M_{wet}}$ is the average mass of individuals in wet treatments.

Effects of soil microbes on plant growth under dry or wet conditions were assessed using the following soil-plant feedback index (Brinkman et al., 2010):

$$Soil{-}plant\ feedback\ index = \frac{M_{live} - \overline{M_{sterile}}}{\overline{M_{sterile}}}$$

where M_{live} is the mass of individuals in live soil treatments, and $\overline{M_{sterile}}$ is the average mass of individuals in sterilized soil treatments.

2.3. Microbial community analysis

Soil samples were collected from all pots at final harvest and sieved (2 mm mesh size). A subsample of soil from each pot was oven-dried (105 °C, 24 h) to determine soil moisture content at harvest. In addition, soil subsamples were taken from all pots with 'live soil' and kept at −20 °C prior to microbial analysis.

Soil microbial communities were determined using phospholipid fatty acid analysis (PLFA) described in Bossio and Scow (1998). In brief, 8 g dry-weight equivalent of fresh soil was used to extract lipids with a one-phase mixture of chloroform, methanol and phosphate buffer (1: 2: 0.8, v/v/v). The lipids were fractioned into neutral, glycol- and polar lipids using solid-phase extraction columns by eluting with chloroform, acetone and methanol respectively. Phospholipids were dissolved in 200 µl hexane containing 19:0 as internal standards after a mild-alkaline methanolysis. The identification and quantitation of PLFAs were conducted using an Agilent 6890 Gas Chromatograph (Agilent Technologies, Palo Alto, CA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE). The fatty acid nomenclature used was described in Frostegård et al. (1993). Total microbial biomass was estimated using the sum concentrations of 13 identified PLFAs. The sum of the following PLFAs was used to measure bacterial biomass: i14:0, a15:0, i15:0, i16:0, 16:1ω7c, a17:0, cy17:0, i17:0, 17:1ω8c, 18:1ω7c, 18:1ω9c and cy19:0. The PLFA 18:2ω6,9c was used as a measure of fungal biomass (Frostegård et al., 2011). PLFA specific to Gram + bacteria (a15:0, i15:0, i16:0, a17:0 and i17:0) and Gram – bacteria (16:1ω7c, cy17:0, 18:1ω7c and cy19:0) were used to represent the bacterial groups (Grayston et al., 2001). The fungi: bacteria ratio and Gram +: Gram − bacteria ratio were calculated to examine changes in microbial community structure.

2.4. Statistical analysis

Plant variables including plant biomass, RGR, root: shoot ratio, SLA, SRL, plant N content and biomass: N ratio were analysed using two-way ANOVA with water and soil treatments as fixed factors. This statistical model was also used to assess treatment effects on soil moisture content. The plant drought response index was analysed using one-way ANOVA with soil treatment as the factor, whereas the soil-plant feedback index was analysed using one-way ANOVA with water treatment as the factor. Principal components analysis (PCA) was conducted to examine the influence of water treatments on microbial community structure, based on percentages of the 13 individual PLFA markers in total PLFA.

Total PLFA, fungi: bacteria and Gram +: Gram − ratios were analysed using one-way ANOVA with water treatment as the factor.

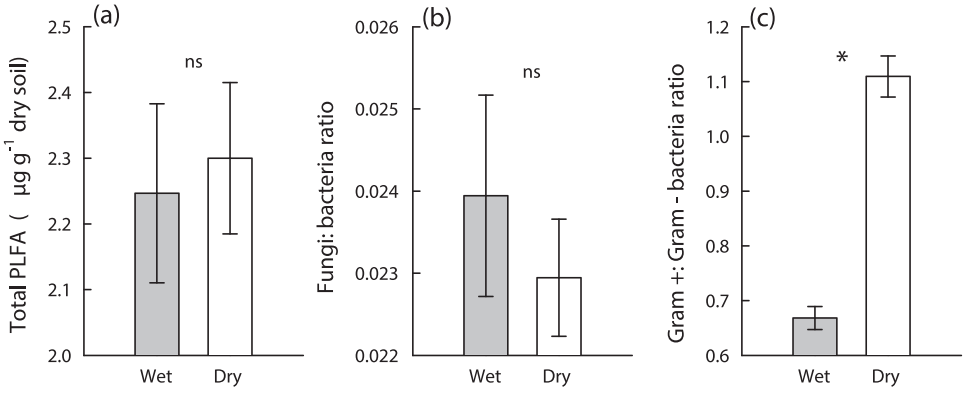

Fig. 4. Effects of water treatments on (a) total PLFA concentrations, (b) fungi: bacteria ratio and (c) Gram +: Gram − ratio of soil microbial communities in live soil treatments. Mean ± SE are shown (n = 6). Differences between wet and dry treatments are indicated (ns, $p > 0.05$; *, $p < 0.05$).

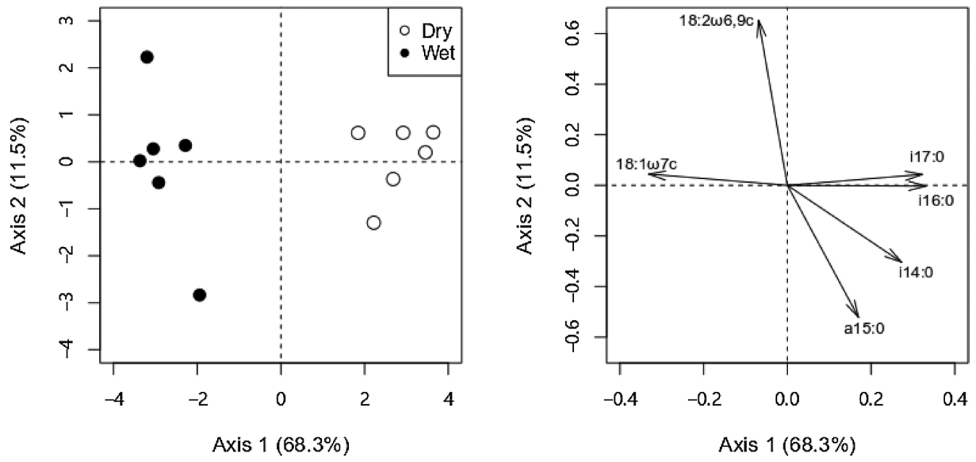

Fig. 5. The first two components and factor loadings from principal components analysis based on soil microbial community structure under dry or wet treatments.

Individual PLFAs that were used for bacteria and fungi were analysed using a mixed-model procedure for split-plot two-way ANOVA, with water treatment as the fixed whole-plot ANOVA, PLFA marker as the fixed sub-plot factor and pot as the random factor (Quinn and Keough, 2002). Differences between treatments were determined using Tukey's honest significant difference post hoc tests. All analyses were conducted using STATGRAPHICS Centurion XVI (StatPoint Technologies, Inc., USA). All data met assumptions of variance homogeneity and residual normality for ANOVA.

3. Results

3.1. Tree seedling growth and plant traits

Plant performance was generally lower in the pots with 'live' versus 'sterile' soil (Table 1, Fig. 1). Reduced water availability also had a negative effect on total seedling biomass, shoot biomass and RGR during the experimental period (Table 1, Fig. 1). These drought-induced reductions were more pronounced in sterile treatments compared to pots with 'live' soil (significant water × soil interactions, Table 1).

Values of root: shoot ratios were higher in dry compared to wet conditions, and also higher in sterile compared to 'live' soil (Table 1, Fig. 1). Unlike root: shoot ratios, specific root length (SRL) only responded to soil treatment, with higher values in sterile compared to 'live' soil (Table 1, Fig. 1). Neither SRL nor root: shoot ratio showed any interaction between water and soil treatments. Specific leaf area (SLA) showed no response to any treatment combination (Table 1, Fig. 1).

Plant N content showed significant responses to both soil and water treatments (Table 1), with higher values in sterile or wet treatments compared to live or dry treatments respectively (Fig. 1). In general, biomass: N ratios were lower in 'live' compared to sterile soil, although the magnitude of decrease depended on water treatments (significant water × soil interaction, Table 1). Negative effects of 'live' soil on biomass: N ratios were greater under wet conditions (−38% versus −11% for wet and dry treatments respectively, Fig. 1).

Plant drought response indices responded to soil treatments at the whole-plant, shoot and root levels, with significantly higher values in the 'live' soil treatments in all cases ($F_{1,10}$ = 12.96, 11.03 and 12.09 respectively, $p < 0.01$ in all cases; Fig. 2). In addition, soil-plant feedback indices were consistently lower in dry compared to wet treatments ($F_{1,10}$ = 10.68 and $p = 0.009$ for whole-individual level, $F_{1,10}$ = 13.44 and $p = 0.004$ for shoot level, $F_{1,10}$ = 8.67 and $p = 0.015$ for root level; Fig. 3).

3.2. Drought effects on soil moisture and soil microbial community structure

Soil moisture content was significantly lower in dry compared to wet treatments (−88% on average, $F_{1,20}$ = 503.11, $p < 0.001$, Fig. S1). Soil moisture content did not show any response to soil treatment or soil × water interactions ($p > 0.05$).

Reduced water availability had no effect on either total PLFA concentrations ($F_{1,10}$ = 0.09, $p = 0.77$, Fig. 4) or the fungi: bacteria ratio ($F_{1,10}$ = 0.49, $p = 0.50$, Fig. 4). However, the Gram +: Gram − bacteria ratio was significantly higher in dry compared to wet treatments ($F_{1,10}$ = 105.53, $p < 0.001$, Fig. 4). Absence of drought effects on total PLFA masked variation in individual PLFA responses (water × PLFA marker interaction, $F_{12,120}$ = 39.98, $p < 0.001$, Fig. S2). Interestingly, PCA analysis provided evidence for differentiation in microbial community structure between the dry and wet treatments (Fig. 5). The first two axes of the PCA analysis accounted for 79.8% of the total variance. Axis one was positively correlated with i16:0 and i17:0 (Gram + bacteria markers) and negatively correlated with 18:1ω7c (a Gram − bacterial group) whereas axis two was positively correlated with 18:2ω6,9c (the fungal biomarker) and negatively correlated with i14:0 and a15:0 (Gram + bacteria). Dry treatments were characterized by high i16:0 and i17:0 and low 18:1ω7c compared to wet treatments (Fig. 5).

4. Discussion

Drought can exert strong impacts on aboveground and belowground processes (Bloor and Bardgett, 2012; Fuchslueger et al., 2014), but the contribution of soil-plant interactions to plant drought resistance remains unclear (de Vries et al., 2012; Delgado-Baquerizo et al., 2017). In this study, we used an innovative experimental approach to investigate the role of soil microbial communities for plant responses to water availability and to provide mechanistic insights into plant drought resistance.

Our first hypothesis was that plant growth would be negatively impacted by the presence of soil microbes. This was supported by our data; seedling biomass was lower in 'live' soil treatments, consistent with previous observations that soil microbes have negative feedback effects on plant growth (van der Putten et al., 2013; Rutten et al., 2016). Microbe-induced decreases in seedling biomass were mirrored by decreases in plant N content, suggesting that observed microbial effects may have been driven by plant-soil competition for nitrogen (Hodge et al., 2000; Wardle et al., 2004). Soil pathogens could also have played a role in depressed plant growth in the presence of soil microbes (Berger et al., 2007), since we observed pathogen-induced leaf spots in 'live' soil.

Seedling ability to maintain high N content and plant biomass in

sterile soil may have been enhanced by the increased allocation to roots and investment in finely-branched root architecture observed in this treatment. We consider that this apparent nutrient effect is unlikely to be an artefact due to soil sterilisation at the start of the experiment (e.g. de Vries et al., 2015) since the volume of soil inoculum was low compared to the other components of the growing medium. In the longer term, seedlings in sterile soil might be expected to show progressive nitrogen limitation as the soil inorganic N supply is depleted; whilst we cannot guarantee that the sterile soil remained free of microorganisms throughout the experiment (due to contamination from air/water), it is reasonable to assume that sterile soil did not have the cohort of microbes associated with efficient N cycling in soil.

Our second hypothesis was that plant drought resistance would be modified in the presence of soil microbes. This too was supported by the data. We found that reduced water availability decreased seedling biomass production and relative growth rates, in line with previous studies (Chaves et al., 2003; Craine et al., 2013). However, drought-induced biomass reductions were significantly lower in 'live' soil treatments, resulting in a less negative value for the plant drought response index in the presence of soil microbes. Plant N content decreased in dry conditions irrespective of soil treatment, likely reflecting decreased soil inorganic N mobility under drought (Xi et al., 2015). Moreover, seedlings in dry conditions increased their investment in roots rather than shoots, consistent with an adaptive response to limiting belowground resources. These shifts in allocation could have significant implications for subsequent plant growth and plant-plant interactions during the rewetting/ drought recovery periods (Hodge and Fitter, 2013).

Our results provide clear evidence that the presence of soil microbes increases drought resistance in *Bauhinia brachycarpa* seedlings. This fits with recent work on temperate tree species, showing positive effects of soil microbial presence on leaf gas exchange and water potential under droughted conditions (Kannenberg and Phillips, 2017). At the same time, we found that soil-plant feedbacks were weaker (i.e. less negative) under dry conditions. Weakened soil-plant feedbacks could reflect an increase in the relative role of positive microbial effects in mediating plant growth under dry conditions (for example, greater reliance on access to water in soil microsites via mycorrhizae (Aroca and Ruiz-Lozano, 2009), and/or a reduction in negative microbial effects such as competition for nitrogen or pathogens (Bloor and Bardgett, 2012; Kaisermann et al., 2017). Weakened soil microbial feedbacks under dry conditions may also arise if plants invest more in defence compounds or allelopathy as resource limitation increases (Smith-Ramesh and Reynolds, 2017). Interestingly, the plant biomass: N ratio showed a greater increase under dry compared to wet conditions when soil microbes were present, suggesting that the weaker soil feedback effects were also associated with a concurrent increase in seedling N use efficiency under dry conditions (Yuan et al., 2006).

Observed drought-induced changes in soil feedbacks were accompanied by an increase in the abundance of Gram+ bacteria within the soil microbial community. Gram+ bacteria are known for their capacity to withstand drought stress, and are often found to increase in abundance under dry conditions (de Vries and Shade, 2013; Fuchslueger et al., 2014, 2016). The increased abundance of Gram+ bacteria reflected compensatory growth by this bacterial group since total microbial biomass was unchanged across water treatments. This supports the idea that slower-growing Gram+ bacteria promote microbial community resistance to climate change-related disturbances (de Vries and Shade, 2013). Importantly, our results indicate that drought alters soil feedback effects due to drought-induced changes in microbial community structure rather than microbial biomass. This agrees with recent work on grass species where legacy effects of drought on soil microbial communities were shown to have knock-on effects on plant-soil feedbacks (Kaisermann et al., 2017). Our results also suggest that soil microbial biomass stability occurs at the expense of soil feedbacks, providing a possible mechanism for trade-offs between plant and microbial biomass stability to drought reported elsewhere (Bloor and Bardgett, 2012).

In the present work, we used field-collected soil conditioned by conspecific adult trees and compared tree seedling responses to drought in 'live' or sterile soil. However, growing evidence suggests that soil feedback effects may be particularly strong in savannah and tropical trees grown in the presence of conspecific soil (Rutten et al., 2016). These negative conspecific effects are often attributed to host-specific pathogens, and are considered to play an important role for plant coexistence by limiting conspecific recruitment close to adults (Janzen-Connell effects, reviewed by Comita et al. (2014)). Where tree seedlings grow in heterospecific soil, it is therefore possible that seedlings may experience less negative soil feedbacks and smaller benefits of drought-induced changes to soil feedbacks in terms of drought resistance. Nevertheless, drought-induced changes in feedback effects may have significant consequences for plant-plant interactions during the drought recovery phase (Kaisermann et al., 2017). In order to better understand the role of soil feedbacks in savannah systems, future studies should address the interactions between drought and soil feedback effects using a greater number of tree species growing in the presence or absence of grasses. Additional insight into the relative importance of microbial groups in mediating plant drought responses may also be gained by screening soil microbial communities with both PLFA and quantitative DNA techniques (Frostegård et al., 2011).

5. Conclusions

To our knowledge, this is the first study to examine the importance of soil microbial presence and soil feedback effects for plant drought resistance. Overall our results suggest that plant drought resistance is mediated by soil-plant feedbacks and drought-induced changes in soil microbial community structure. Positive effects of soil microbial presence on plant drought resistance were enhanced by weakened soil-plant feedbacks and shifts in microbial community structure rather than changes in microbial biomass production. In particular, compensatory growth by Gram+ bacteria appeared to play a key role in microbe-mediated mechanisms of plant drought resistance. This underlines the importance of understanding how climate change impacts soil microbial communities and plant-soil interactions in order to improve predictions of plant responses to future climatic conditions (Kannenberg and Phillips, 2017; Fry et al., 2018). Further work is needed to test the generality of our findings with other plant species/functional groups, and to determine the relative importance of plant and soil microbial traits for plant drought resistance.

Author statement

We confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome. All authors have read and approved the manuscript being submitted. This article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Author contribution

NX conceived the ideas and designed methodology; NX collected and analysed the data; NX, CC and JMGB interpreted the results and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

The authors thank Xueqiao Zhang and Yan Wang for assistance with plant and soil measurements, thank Mareike Roeder for seed collection, and thank Xiaojing Wang for greenhouse support. This study was

funded by the National Natural Science Foundation of China (31600342 to NX, 31570426 and 31622014 to CC).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2018.08.013.

References

Aroca, R., Ruiz-Lozano, J.M., 2009. Induction of plant tolerance to semi-arid environments by beneficial soil microorganisms – a review. In: Lichtfouse, E. (Ed.), Climate Change, Intercropping, Pest Control and Beneficial Microorganisms. Springer Netherlands, Dordrecht, pp. 121–135.

Baudena, M., Dekker, S.C., van Bodegom, P.M., Cuesta, B., Higgins, S.I., Lehsten, V., et al., 2015. Forests, savannas, and grasslands: bridging the knowledge gap between ecology and dynamic global vegetation models. Biogeosciences 12, 1833–1848.

Baxendale, C., Orwin, K.H., Poly, F., Pommier, T., Bardgett, R.D., 2014. Are plant–soil feedback responses explained by plant traits? New Phytol. 204, 408–423. https://doi.org/10.1111/nph.12915.

Berger, S., Sinha, A.K., Roitsch, T., 2007. Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. J. Exp. Bot. 58, 4019–4026. https://doi.org/10.1093/jxb/erm298.

Bloor, J.M.G., Bardgett, R.D., 2012. Stability of above-ground and below-ground processes to extreme drought in model grassland ecosystems: interactions with plant species diversity and soil nitrogen availability. Perspect. Plant Ecol. Evol. Syst. 14, 193–204. https://doi.org/10.1016/j.ppees.2011.12.001.

Bloor, J.M.G., Niboyet, A., Leadley, P.W., Barthes, L., 2009. CO_2 and inorganic N supply modify competition for N between co-occurring grass plants, tree seedlings and soil microorganisms. Soil Biol. Biochem. 41, 544–552. https://doi.org/10.1016/j.soilbio.2008.12.013.

Bond, W.J., 2008. What limits trees in C_4 grasslands and savannas? Ann. Rev. Ecol. Evol. Syst. 39, 641–659. https://doi.org/10.1146/annurev.ecolsys.110707.173411.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb. Ecol. 35, 265–278. https://doi.org/10.1007/s002489900082.

Brinkman, E., Van der Putten, W.H., Bakker, E.-J., Verhoeven, K.J.F., 2010. Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. J. Ecol. 98, 1063–1073. https://doi.org/10.1111/j.1365-2745.2010.01695.x.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought - from genes to the whole plant. Funct. Plant Biol. 30, 239–264. https://doi.org/10.1071/fp02076.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., et al., 2014. Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. J. Ecol. 102, 845–856. https://doi.org/10.1111/1365-2745.12232.

Cortois, R., Schröder-Georgi, T., Weigelt, A., van der Putten, W.H., De Deyn, G.B., 2016. Plant–soil feedbacks: role of plant functional group and plant traits. J. Ecol. 104, 1608–1617. https://doi.org/10.1111/1365-2745.12643.

Craine, J.M., Ocheltree, T.W., Nippert, J.B., Towne, E.G., Skibbe, A.M., Kembel, S.W., Fargione, J.E., 2013. Global diversity of drought tolerance and grassland climate-change resilience. Nat. Clim. Change 3, 63–67. https://doi.org/10.1038/NCLIMATE1634.

de Vries, F., Shade, A., 2013. Controls on soil microbial community stability under climate change. Front. Microbiol. 4, 265. https://doi.org/10.3389/fmicb.2013.00265.

de Vries, F.T., Liiri, M.E., Bjørnlund, L., Setälä, H.M., Christensen, S., Bardgett, R.D., 2012. Legacy effects of drought on plant growth and the soil food web. Oecologia 170, 821–833. https://doi.org/10.1007/s00442-012-2331-y.

de Vries, F.T., Jørgensen, H., Hedlund, K., Bardgett, R.D., 2015. Disentangling plant and soil microbial controls on carbon and nitrogen loss in grassland mesocosms. J. Ecol. 103, 629–640. https://doi.org/10.1111/1365-2745.12383.

Delgado-Baquerizo, M., Eldridge, D.J., Ochoa, V., Gozalo, B., Singh, B.K., Maestre, F.T., 2017. Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. Ecol. Lett. 20, 1295–1305. https://doi.org/10.1111/ele.12826.

Druce, D.J., Shannon, G., Page, B.R., Grant, R., Slotow, R., 2008. Ecological thresholds in the savanna landscape: developing a protocol for monitoring the change in composition and utilisation of large trees. PLoS One 3, e3979. https://doi.org/10.1371/journal.pone.0003979.

Fargione, J., Tilman, D., 2006. Plant species traits and capacity for resource reduction predict yield and abundance under competition in nitrogen-limited grassland. Funct. Ecol. 20, 533–540. https://doi.org/10.1111/j.1365-2435.2006.01116.x.

Friesen, M.L., Porter, S.S., Stark, S.C., von Wettberg, E.J., Sachs, J.L., Martinez-Romero, E., 2011. Microbially mediated plant functional traits. Ann. Rev. Ecol. Evol. Syst. 42, 23–46. https://doi.org/10.1146/annurev-ecolsys-102710-145039.

Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730. https://doi.org/10.1016/0038-0717(93)90113-P.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621–1625. https://doi.org/10.1016/j.soilbio.2010.11.021.

Fry, E., Johnson, G., Hall, A., Pritchard, W., Bullock, J., Bardgett, R., 2018. Drought neutralises plant-soil feedback of two mesic grassland forbs. Oecologia 186, 1113–1125. https://doi.org/10.1007/s00442-018-4082-x.

Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., Richter, A., 2014. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytol. 201, 916–927. https://doi.org/10.1111/nph.12569.

Fuchslueger, L., Bahn, M., Hasibeder, R., Kienzl, S., Fritz, K., Schmitt, M., et al., 2016. Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. J. Ecol. 104, 1453–1465. https://doi.org/10.1111/1365-2745.12593.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biol. Biochem. 33, 533–551. https://doi.org/10.1016/S0038-0717(00)00194-2.

Hodge, A., Fitter, A.H., 2013. Microbial mediation of plant competition and community structure. Funct. Ecol. 27, 865–875. https://doi.org/10.1111/1365-2435.12002.

Hodge, A., Robinson, D., Fitter, A., 2000. Are microorganisms more effective than plants at competing for nitrogen? Trends Plant Sci. 5, 304–308. https://doi.org/10.1016/s1360-1385(00)01656-3.

Hoffmann, W.A., Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. Ann. Bot. 90, 37–42. https://doi.org/10.1093/aob/mcf140.

IPCC, 2007. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York, USA, pp. 1–18 2007.

Kaisermann, A., de Vries, F.T., Griffiths, R.I., Bardgett, R.D., 2017. Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. New Phytol. 215, 1413–1424. https://doi.org/10.1111/nph.14661.

Kannenberg, S.A., Phillips, R.P., 2017. Soil microbial communities buffer physiological responses to drought stress in three hardwood species. Oecologia 183, 631–641. https://doi.org/10.1007/s00442-016-3783-2.

Karlowsky, S., Augusti, A., Ingrisch, J., Hasibeder, R., Lange, M., Lavorel, S., et al., 2017. Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. J. Ecol. 00, 1–14. https://doi.org/10.1111/1365-2745.12910.

Kulmatiski, A., Beard, K.H., Stevens, J.R., Cobbold, S.M., 2008. Plant-soil feedbacks: a meta-analytical review. Ecol. Lett. 11, 980–992. https://doi.org/10.1111/j.1461-0248.2008.01209.x.

Lau, J.A., Lennon, J.T., 2011. Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. New Phytol. 192, 215–224. https://doi.org/10.1111/j.1469-8137.2011.03790.x.

Lobet, G., Pagès, L., Draye, X., 2011. A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol. 157, 29–39. https://doi.org/10.1104/pp.111.179895.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938. https://doi.org/10.1890/11-0026.1.

Pérez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al., 2013. New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 61, 167–234. https://doi.org/10.1071/bt12225.

Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, New York, USA.

Rutten, G., Prati, D., Hemp, A., Fischer, M., 2016. Plant–soil feedback in East-African savanna trees. Ecology 97, 294–301. https://doi.org/10.1890/15-1316.1.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394. https://doi.org/10.1890/06-0219.

Scholes, R.J., Archer, S.R., 1997. Tree-grass interactions in savannas. Annu. Rev. Ecol. Syst. 28, 517–544. https://doi.org/10.1146/annurev.ecolsys.28.1.517.

Smith-Ramesh, L.M., Reynolds, H.L., 2017. The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. J. Veg. Sci. 28, 484–494. https://doi.org/10.1111/jvs.12519.

van der Molen, M.K., Dolman, A.J., Ciais, P., Eglin, T., Gobron, N., Law, B.E., et al., 2011. Drought and ecosystem carbon cycling. Agric. For. Meteorol. 151, 765–773. https://doi.org/10.1016/j.agrformet.2011.01.018.

van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., et al., 2013. Plant–soil feedbacks: the past, the present and future challenges. J. Ecol. 101, 265–276. https://doi.org/10.1111/1365-2745.12054.

van der Putten, W.H., Bradford, M.A., Pernilla Brinkman, E., van de Voorde, T.F.J., Veen, G.F., 2016. Where, when and how plant–soil feedback matters in a changing world. Funct. Ecol. 30, 1109–1121. https://doi.org/10.1111/1365-2435.12657.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875.

Williams, M.A., Rice, C.W., 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Appl. Soil Ecol. 35, 535–545. https://doi.org/10.1016/j.apsoil.2006.09.014.

Xi, N., Carrère, P., Bloor, J.M.G., 2015. Plant community responses to precipitation and spatial pattern of nitrogen supply in an experimental grassland ecosystem. Oecologia 178, 329–338.

Yang, J., Kloepper, J.W., Ryu, C.-M., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14, 1–4. https://doi.org/10.1016/j.tplants.2008.10.004.

Yuan, Z.-Y., Li, L.-H., Han, X.-G., Chen, S.-P., Wang, Z.-W., Chen, Q.-S., Bai, W.-M., 2006. Nitrogen response efficiency increased monotonically with decreasing soil resource availability: a case study from a semiarid grassland in northern China. Oecologia 148, 564–572. https://doi.org/10.1007/s00442-006-0409-0.

Zwicke, M., Picon-Cochard, C., Morvan-Bertrand, A., Prud'homme, M.-P., Volaire, F., 2015. What functional strategies drive drought survival and recovery of perennial species from upland grassland? Ann. Bot. 116, 1001–1015. https://doi.org/10.1093/aob/mcv037.